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#19

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Date: June 13, 2003 By: Carol A. See
Carol A. See

PATENT
Docket No. GC527C2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	
D. A. Estell *et al.*	Group Art Unit: 1644
Serial No.: 09/677,822	Examiner: Saunders, D.
Filed: October 2, 2000	
For: Proteins Producing an Altered Immunogenic Response and Methods of Making and Using the Same	

DECLARATION OF FIONA HARDING UNDER 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Fiona Harding, am a co-inventor of the subject matter embodied in the above-identified patent application.

2. I have read and understand the above-identified patent application, which was filed on October 2, 2000; the priority patent applications Application Serial Number 09/500,135, filed February 8, 2000, and Application Serial Number 09/060,872, filed April 15, 1998; all of the Claims as amended and filed in the "Amendment and Response to Office Action" filed herewith; the Office Action from the U.S. Patent & Trademark Office, mailed January 14, 2003; and the references by Landry (WO 99/06061; published February 11, 1999), and Mouritsen (WO 95/05849; published March 2, 1995).

3. The work that is the subject of the pending Claims and that is described in paragraphs 4 to 11 below, was performed in this country by me or under my supervision.

4. Prior to February, 1999, I successfully carried out assay experiments as described in the present patent application, in which the amino acid sequence of a T-cell

epitope peptide was modified to increase the magnitude of the induced proliferative response. A peripheral blood (PBMC) sample from a Genencor employee who was verified by Genencor's Environmental Health and Safety department as sensitized to *B. lentus* subtilisin was drawn by the Stanford University Blood Center. Monocytes from the PBMC sample were cultured with GM-CSF and IL-4 for 5 days in order to cause the differentiation of dendritic cells (DC). IL-1 and TNF-alpha were subsequently added, and the DC cultures were incubated for another 2 days. The final DC cultures were harvested on day 7. On day 7, CD4+ T cells from the donor PBMC sample were isolated from frozen aliquots.

5. Peptides encompassing the amino acids 160-174 from *B. lentus* subtilisin, and a series of alanine scan peptide variants, were purchased from Mimotopes.

6. CD4+ T cells and DC from the sensitized donor were co-cultured with either the unmodified parent peptide, or the alanine substituted variant peptides. Cultures were incubated for 5 days. On day 5, 0.5 uCi of tritiated thymidine was added to each well of the cell culture. On day 6, the cell cultures were harvested to glass fiber mats, and incorporated tritiated thymidine was measured.

7. This donor had been previously shown to respond to the amino acid 160-174 region of *B. lentus* subtilisin by mounting a CD4+ T cell response. In this experiment, the donor again responded to the unmodified amino acid 160-174 peptide. The stimulation index of the proliferative response (experimental cpm divided by control well cpm) was about 7.

8. Responses to the alanine scan peptides were tabulated. Many of the alanine substituted peptides have no effect on the proliferative response (*i.e.*, the magnitude of the stimulation index to the variant peptide was approximately the same as the response to the unmodified parent peptide). Alanine changed peptides at some of the positions (R, Y, N) had a deleterious effect on the induction of a proliferation response. However, alanine substitutions at both positions #12 and #5 in the peptide resulted in proliferative responses that were more robust than the response to the unmodified parent peptide.

9. Please see Figure 11 in the application as filed, which shows the increased proliferative response to variant peptide carrying changes #5 and #12 as compared to the parent, unmodified peptide sequence.

10. In conclusion, this experiment shows that the modification of a T-cell epitope peptide sequence can result in an increased proliferative response to the polypeptide.

11. These experiments are described on pages 89-99 of my laboratory notebook number 1471, attached hereto at Tab 1.

The undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 19 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom

Dated: 12 June 2003

Signed: Fiona Harding
FIONA HARDING

Project No.______ 14 Oct 97

TITLE M29704 (directed donation) Book No.______ 87

From Page No.__

(1) Directed donation by a sensitized individual. Buffy prepared by Stanford Blood Bank

(2) Ficoll, wash, count
(205 x 10 x 9): 1×10^9 total.

(3) Prepare EBV transformed B cells: 2.5 ml B95 SN 2.5 ml 10% FCS RPMI + 10^7 cells. 4:30 pm

(4) Set up DC in a 6 well plate: 4 wells IL-4 / GM-CSF 3:50

(5) 1° Response to GG36: Titer GG36 40 µg/ml and down. TT 40 µg/ml & down.
AIMV. 2×10^5/well. KLH, Amylase @ 40 µg/ml and down.

(6) Set up DC 10^8 in a T75 for GG36 peptide test 3:40 pm

(7) Freeze all other PBMC: 2×10^7/ml in FCS/10% Fresh DMSO.

(8) TNF/IL-1a to DC cultures 20 Oct 97. Pulse 1° Response

(9) Harvest 1° Response

(10) in Situ Mitomycin C. 1½ hrs @ 37°C. wash DC, cells w/ wells. Return washed cell wells to wells.
M29704 DC Count: 100 x 10 ml = 1×10^7 total.

(11) CD4+ Select Columns lot # B2797B
10^8 cells/column. 3 columns. Ran perfectly!
pooled 75 x 10 750 x 10 7.5×10^7 total cells.
37.5 mls for 2×10^6/ml.

(12) 6×10^6 CD4+ T + 1 well of 6 well plate w/ 40 µg/ml GG36
and 2° DCs + 10 ng/ml rh IL-7
6×10^6 CD4+ T + 1 well of 6 well plate w/ ~~40~~ 10 µg/ml
Amylase and 2° DCs + 10 ng/ml rh IL-7

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Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
[signature]	05/3/99	Recorded by [signature]	14 Oct 97

TITLE directed donation M29704 1° resp. Project No. Book No. 90

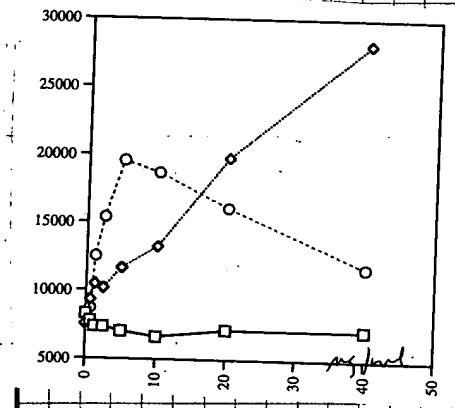
1450-21.10-01

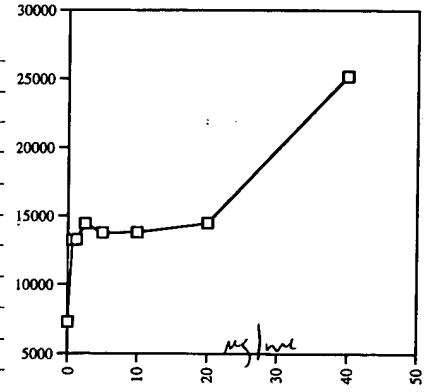
From Page No. 87

1° Response by whole PBMC to protein ags.

M29704 (directed donation) Primary response to GG36, Tetanus, KLH and Amylase

CCPM1	21-Oct-97											
	1	2	3	4	5	6	7	8	9	10	11	12
A	7104.6	7404.3	6958.2	25880.9	25220.3	24517.1	31377	28978.5	28091.2	12018.1	11744.5	11469.2
B	7779.9	7019.2	6869.6	13417.4	13552.9	16455.5	20676.2	17894.5	20791	16581.1	16170.3	15684.9
C	8358.7	6747.2	6767.6	13806	15114.1	12490.3	12228.5	12183.4	15276.8	17891.9	17613.8	20705.8
D	8778	7270.4	7023	14192.6	12367.9	14681.8	11456.8	11672.2	11806.5	20535.2	19774.8	18373.6
E	7293.1	7730	7043.5	16049.3	14266	12966.8	10022.8	9714	10775.8	16398.9	15635.1	14084.8
F	7444.3	6935.3	7870.3	13798.1	13422.5	12560.7	10663.2	10721.1	9960.7	11318.7	13215	13003.6
G	8315.7	6842	8297.3	11205.9	14388.1	14083.5	9559.1	10971.3	7427.8	8775.1	8093.7	8247.4
H	8865.7	7672.1	8276.8	8469.4	8169.5	7206	7536.3	7706.6	7338.7	8336.7	8904.2	8797.8
#PE			GG36			KLH			KLH			Amylase
		40	7155.0			25208.1			28148.9			11743.3
		20	7156.2			14475.3			19787.2			18112.1
		10	6824.5			13803.5			13229.6			18670.5
		5	7023.8			13747.4			11645.2			19581.2
		2.5	7355.5			14427.4			10170.9			15373.3
		1.25	7350.0			13280.4			10448.3			12512.4
		0.63	7751.7			13225.2			9319.4			8705.4
		0	8271.6			7289.0			7527.2			8348.2





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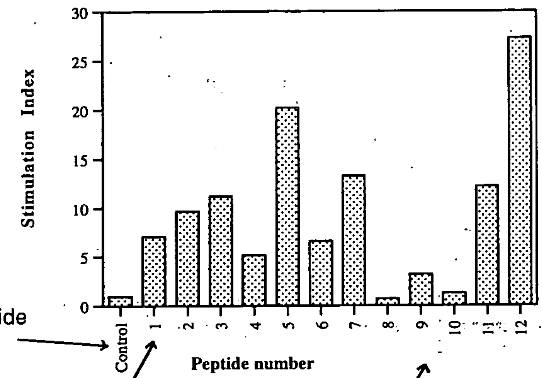
Witnessed & Understood by me,	Date	Invented by	Date
	05/3/99	Recorded by	23 Oct 97

92.5

M29704 directed donation
Response to alanine substituted EP-2 peptides



Yikes!

No peptide added

Unsubstituted EP2

We like this a lot

FEB 05 '98 03:06PM STANFORD BLOOD CNTR P.2/3

HLA TEST REPORT

STANFORD HISTOCOMPATIBILITY LABORATORY
STANFORD MEDICAL SCHOOL BLOOD CENTER
800 Welch Road - Palo Alto, CA 94303
Director: F Carl Grumet, MD
Co-Director: Alan Ting, PhD

Questions re Billing: (650) 723-7994
Technical/Medical: (650) 723-5548

CLIA # 05D0716472
ASHI # 6-CA-08-2

PATIENT: M29704 RESEARCH
MEDICAL RECORD # - DATE OF BIRTH: / /
REQUESTING PHYSICIAN: FIONA HARDING
CATEGORY: GENENCOR INTERNATIONAL
SAMPLE DATE: 01/30/98
TEST DATE: 02/04/98
DATE OF REPORT: 02/04/98

HLA-DR: DRB1*01 DRB1*15

DRB, DQB1 performed by PCR-SSP, PCR-SSOP, and/or Sequencing
Cw performed by PCR-SSP, PCR-SSOP, Sequencing, and/or Serology
X = Undetected antigen/allele, or homozygosity
@ = See Comment

Fiona Harding

TO: FIONA HARDING
GENENCOR INTERNATIONAL
925 PAGE MILL RD
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FAX:415-8456509
PHONE:415-846-7561

Susan Dunn HT(ABHI)
Report Reviewed By

2/5/98
Date

Date Faxed 2-5-98 1505
By
TS
MOTC
Apheresis
845-6509

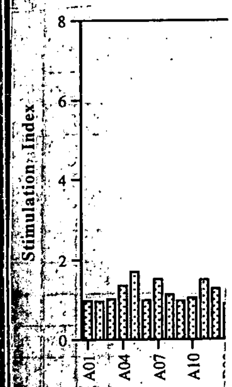
TITLE M29704

From Page No. 90

(13) Ba...

(14) Collect
...-2 ...

(15) harvest



	M29704 d...	
CCPM1	28-Oc...	
A		
B	44	
C	83	
D	88	
E		
F	8	
G		
H		
SPE		
Ave. of tripl.	71	
SI		
	10	
	control	

Witnessed & Understo...

Yikes!

From Page No. 90

(13) Decant DC from frozen aliquot of cells. One T75 flask

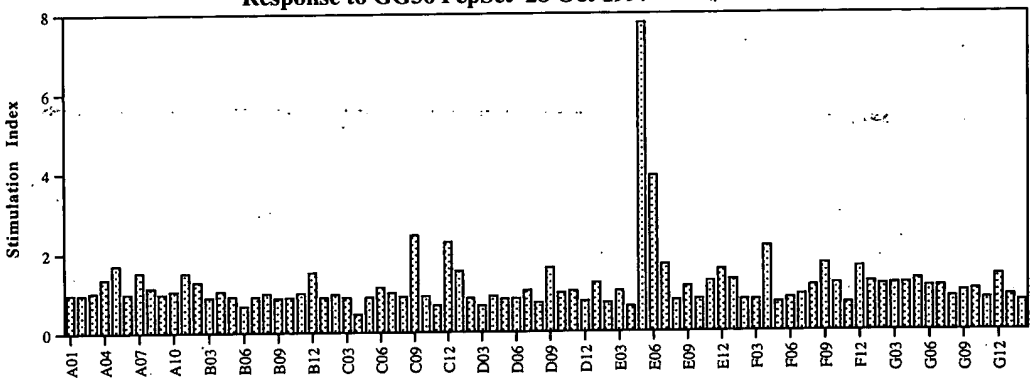
(14) Collect Activated cells. Wash and Count. Plate in AIMV + 10U/ml IL-2 and 10ng/ml rh-IL-7

GG36 pipred: 3.8×10^6 total

Amylin pipred: 3.1×10^6 total

(15) Harvest CFSE/TT/DC Response to PepSet and Substitutes EP2

M29704 directed donation
Response to GG36 PepSet 28 Oct 1997 1450PRM28-10-1



1450-28.10-02

M29704 directed donation response to substituted EP2 peptides.												
CCPM1	28-Oct-97											
	1	2	3	4	5	6	7	8	9	10	11	12
A	4,1	14,3	0	6,1	18,3	8,2	14,3	8,2	4,1	8,2	4,1	4,1
B	441,1	328,8	118,5	218,5	2085,5	1070,2	93,9	63,3	137,3	100,4	2612,8	4008,5
C	833,3	1170,3	851,7	363,5	3458,3	604,5	134,8	63,3	737,6	155,7	639,2	311,4
D	881,9	1452,2	2434,8	990,8	575,9	330,9	3801,6	79,6	90,1	127	452,8	3963,4
E	6,1	10,2	10,2	10,2	10	4	2	4	8,1	2	2	4,1
F	81,4	81,4	140,3	13171,8	5734,4	5770,4	4	4	2	2	2	0
G	4,1	10,2	4,1	8,1	10	16	8	0	2	2	4,1	6,1
H	4,1	2	2	0	0	4	0	6	8,1	4,1	0	4,1
#E												
Ave of trip.	712,1	883,8	1135,0	523,5	2039,9	668,5	1343,4	68,7	321,7	127,7	1234,9	2761,1
R	7,1	9,7	11,2	5,2	20,2	6,6	13,3	0,7	3,2	1,3	12,2	27,3
	101,0		8225,5									
	control		TT									

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Witnessed & Understood by me, Date 05/3/99 Invented by Date 28 OCT 97 Recorded by

[redacted]

TITLE M24704 Project No. ______ Book No. ______

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(16) Second set of DC. MitoC in-situ 1 hr. Collect, Count

3.5 $\times 10^6$ total.

(17) Collect activated cells. wash. ficoll. Count.

GK36: 3.0 $\times 10^6$ total

Amylase: 2.14 $\times 10^6$ total.

(18) Plate 5 $\times 10^5$/well DC.

10^6/well GK36 — 40 µg/ml GK36

7 $\times 10^5$/well Amylase. — 10 µg/ml Amylase.

in 2 ml. 24 well plate. AIMV.

(19) 11 NOV 97: Cells grew very well, then crashed. Today look mostly dead.

ficoll, Count:

GK36: 1.2 $\times 10^5$ total

Amylase: 2.8 $\times 10^5$ total.

(20) MitoC treat EBV trsf'd B cell. 45' 100 µg/ml MitoC.

wash well. 6 $\times 10^5$ total.

(21) 48 well plate. 3 wells each. 10^5 EBV B cells/well.

12 Nov: GK36 look to be responding to EBV/Ag. Amylase cultures not so good.

(22) 13 Nov 97: Add 10 U/ml IL-2 and 100 ng/ml IL-7 to all GK36 primed wells

To Page No. ______

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	05/13/99	Recorded by [signature]	4 Nov 97